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Research Article

Copper Nanoparticles Synthesized from *Cinnamomum zeylanicum* and its Antibacterial Activity

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Abstract: Nanoparticles of copper have superior properties as compared to the bulk copper material. *Cinnamomum zeylanicum* has been used to synthesise copper nanoparticles in the present study as it is nontoxic and cheap. The prepared copper nanoparticles were characterized by UV– V spectrophotometer showing a typical resonance at about 631 nm which is specific for CuNPs. Fourier transform infrared spectroscopy indicates that oxygen-containing functional groups in the *C zeylanicum* are involved in the nanoparticle synthesis reaction. High-resolution transmission electron microscopy (HRTEM) was also used to confirm that CuNPs are spherical shape. The antimicrobial activity was carried out against *E-coli, Enterobacteria, Stephylococcus aureus, Bascillus* to confirm that these particles may act as antimicrobial agents.

Keywords: Copper nanoparticles; C zeylanicum; antimicrobial activity

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Introduction

The remarkable physical and chemical properties of metal nanoparticles have attracted considerable attention in recent years. These particles are useful for photovoltaic cells, optical and biological sensors, conductive materials, coating formulations and quantum size effect. Different researchers have synthesized different nanoparticles and among these copper nanoparticles are cheap and scalable. They have also some of the useful properties that can be used to treat various diseases at cheaper rates. There are several methods in practice to synthesis copper nanoparticles that include ultrasonic-chemical [1], electrolysis [2], sol-gel [3], inverse microemulsion [4], chemical reduction [5], microwave irradiation [6], bio synthesis [7]. Synthesis of copper nanoparticles from biological compounds is an interesting option and is less costly, nontoxic and eco-friendly in nature.

At present scenario we were synthesized ultra small copper nanoparticles (CuNPs) by using *C zeylanicum* powder as a reducing and capping agent at room temperature. *C zeylanicum* contains about 11% water, 81% carbohydrates, 4% protein, and 1% fat. It is a rich source of vitamin K, calcium and iron, while providing moderate amounts of vitamin B6, vitamin E, magnesium, and zinc. Cinnamon, according to traditional Chinese medicine has been used as a neuroprotective agent [8]and for the treatment of diabetes[9]. It is also used as a health-promoting agent for the treatment of inflammation, gastrointestinal disorders and urinary infections [10-11]. Further, the bark is used as antimicrobial agent, especially antibacterial activity. Infection due to micro organisms is a cause of morbidity and mortality and there were more than 55 million deaths worldwide and most of them succumbed due to infection[12]. At the same time the constant use of antibiotics has lead to the antibiotic resistance of microorganisms along with other side effects [13]. Therefore, synthesis of cupper nanoparticles from cinnamon might act against these resistant microorganisms with immediate effect. The novelty of this study is to investigate the efficiency of cinnamon Copper nanoparticles as antibacterial agents.

Materials and methods

Plant material

Bark of *C zeylanicum* was purchased from the local market in Gwalior, Madhya Pradesh. Preparation of *C zeylanicum* and synthesis of CuNPs was extracted at room temperature by squeezing the prewashed *C zeylanicum* bark and filtered through muslin cloth. The known volume of filtered extract of *C zeylanicum* was added to $CuSO_45H_2O$ solution (100 mM) prepared in sterilized distilled water. The solution was mixed thoroughly, poured into an aluminium vessel for the reaction and gradually heated to boiling (60–100 ^oC).

Detection and characterization of CuNPs Visual observation

The primary detection was carried out by visual observation. The change in color of the precursor solution from blue to pale-yellow or colourless with time and deposition of reddish shiny brown coloured precipitation on the inner side of the vessel provided is an evidence of CuNPs synthesis.

UV- Visible Spectroscopy

The electronic spectra of biosynthesized CuNPs were recorded on Perkin – Elmer UV lambda 25 at Sophisticated Analytical Instrumentation Lab in PC Ray Research Lab of ITM University, Gwalior.

FTIR

FTIR spectra were recorded using KBr pellets on Perkin Elmer RX – 1 in the range 4000 – 400/cm region in PC Ray Research Lab of ITM University, Gwalior.

Transmission Electronic Microscope

TEM was recorded on TEM SX100 (Cameca) at SAIF, AIIMS, New Delhi with magnification ranging 50 to 10^6.

Antibacterial activity

The test cultures were collected from Microbiology Laboratory, Department of Life Science, ITM University, Gwalior. The in-vitro antibacterial activity of the synthesized sample was evaluated against two gram positive bacteria *Staphylococcus aureus* and *B.Subtilis* and two gram negative bacteria *E- coli*, *Enterobacteriaceae* family against the standard antibiotic drug Streptomycin and methanol as control. The effect of our synthesized compound on the several bacterial strains was assayed by Agar well diffusion method. The minimum concentration of the sample to inhibit the microorganisms was determined by micro dilution method using sample diluted in sterile nutrient broth. Positive test results were scored when a zone of inhibition was observed around the well after the incubation period.

Results and discussion

The emergence of nano science and nano technology in the last decade presents opportunity for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. The antibacterial activity of biosynthesized copper nanoparticles was carried out on four human pathogens that is two gram positive bacteria *S aureus* and *B. Subtilis* and two gram negative bacteria *E-coli*, *Entero bacteria* family by Agar disc diffusion method at 1µg/ml concentration. Streptomycin 1µg/ml was used as positive control. Biosynthesized CuNPs showed clear zone of inhibition as indicated in table 1 and Fig. 1.

Table1 Antibacterial activity of biosynthesized *C zeylanicum* CuNPs

Organism	Type of bacteria	Zone of inhibition (mm) *by biosynthesized CuNPs*	Antibiotic Streptomycin (mm) + Control
E-Colli	Gram Negative Bacilli	25	33
Enterobacteria	Gram Negative Bacilli	19	25
Stephylococcus Aureus	Gram Positive Bacilli	21	25.2
Bascillus Subtilis	Gram Positive Bacilli	18	24

The presence of an inhibition clearly indicates the mechanism of biocidal action of nanoparticles that involves the disruption of the membranes. Extent of inhibition depends on the concentration of nanoparticles as well as on the initial bacterial concentration. The reason could be that the smaller size of the particles which gets tightly adsorbed on the surface of the bacterial cells so as to disrupt the membrane; thereby leading to the leakage of intracellular components, thus killing the bacterial cells [14]. Another proposed mechanism involves the association of copper with sulphydyl groups (-S-H-) on the cell wall to form R-S-S-R bonds, thereby blocking respiration and causing cell death [15].

From the result of zone of inhibition values indicated in table 4.4 proves that biosynthesized copper nanoparticles exhibits moderate antibacterial activity against gram negative bacterium. The interaction between Gram-positive bacteria and nanoparticles was stronger than that of Gram-negative bacteria because of the difference in cell walls between Gram positive and Gram negative bacteria.

Spectroscopic Characterization of Biosynthesized Copper Nanoparticles

I. UV- Visible spectroscopy

Nano sized particles exhibit unique optical properties having an exponential decay. UV- Visible absorbance spectroscopy has proved to be a very useful technique for studying metal nanoparticles because the peak positions and shapes are sensitive to particle size. The surface Plasmon peak of Cu NPs has been reported to appear at 252.55 nm, which confirms the formation of Cu NPs (Fig. 2). A blue shift the wave length from 233.67 to 252.55 nm was observed with the increase in the amount of extract. This shift can be explained on the basis of increased nucleation rate due to greater amount of Cu^{2+} ions reduced and generation of nanoparticles in the solution. Reddish brown colour was observed for the optimum amount of extract and precursor producing greatest number of copper nanoparticles in the medium.

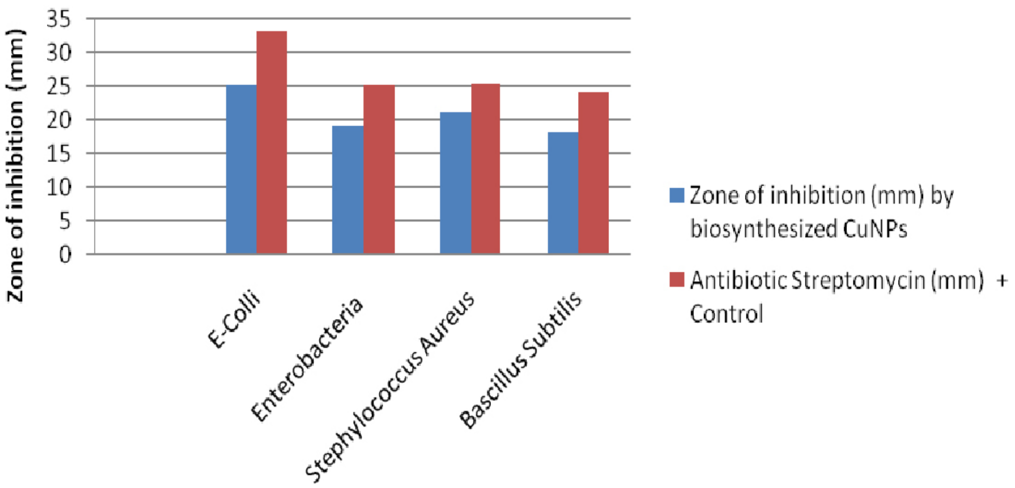

Fig. 1 Antibacterial activity of biosynthesized C *zeylanicum* CuNPs

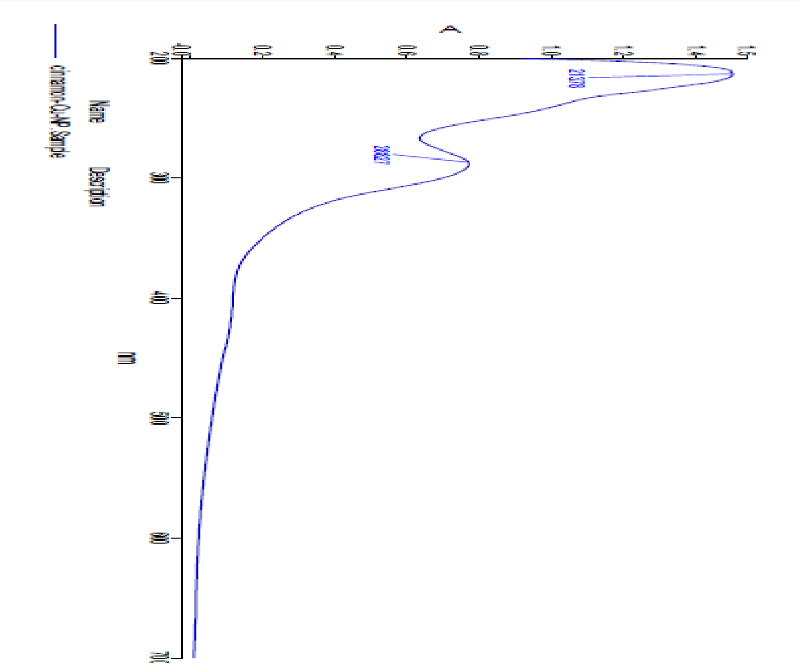

Fig. 2 UV – cinnamon extract and cinnamon copper nanoparticles of 1:4 ratio is given below in Fig. 3.

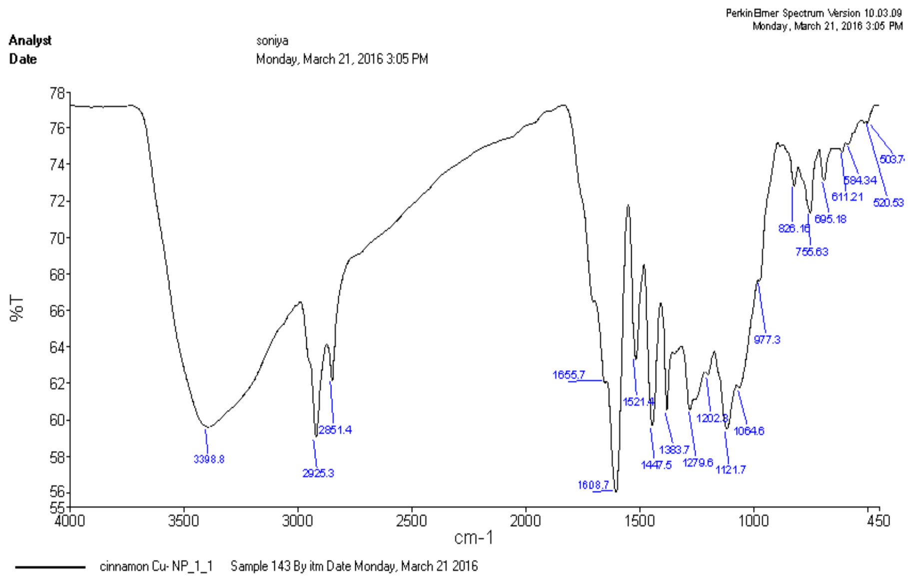

Fig. 3 FTIR Spectra of Copper nano particles obtained from cinnamon bark extract

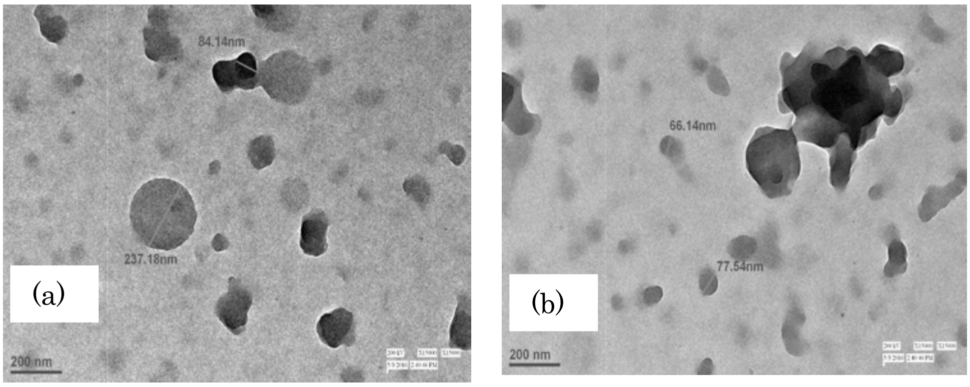

Fig. 4 (a) and (b) showing the TEM images of CuNPs. The size of the CuNps is found to be 66.14 nm.

II. FT - IR Analysis

FTIR measurements of both the methanolic cinnamon extract and the synthesized dried CuNPs were carried out to identify the possible biomolecules responsible for the reduction, capping and efficient stabilization of the bioreduced CuNPs. FTIR spectra of CuNPs in KBr pallets at moderate scanning speed were made. The spectra of $CuSO_4$, cinnamon extract and cinnamon copper nanoparticles of 1:4 ratio is given below in Fig. 3.

III. Transmission Electronic Microscopy

TEM images confirmed that the metal nanoparticles are in nano range and they are approximately spherical in shape. TEM images are shown in Fig. 4 (a) and (b).

Conclusions

Use of *C zeylanicum* for the synthesis of CuNPs is a novel step towards the biogenic synthesis of CuNPs. It is an ecofriendly, non-toxic and rapid approach for antibacterial activity. Further, the use of *C zeylanicum* CuNPs against different human and plant pathogens confirmed its effectiveness against wide range of microorganisms. Due to the antimicrobial property of *C zeylanicum* CuNPs, it can be used in different formulations which could protect humans from the diseases caused by these pathogens. Finally, and most important is that it is a cost effective approach as raw materials involved in the synthesis are very cheap.

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